

sequences, CELC30H7_2, CELC06A6_3, CELF42G8_3, S57942, ER72_CAEEL, CELC07A12_3, CEH06O01_4 and P_R51696.

EXAMPLE 50: Isolation of cDNA Clones Encoding Human PRO181

A cDNA sequence isolated in the amylase screen described in Example 2 above was found, by BLAST and FastA sequence alignment, to have sequence homology to a nucleotide sequence encoding the cornichon protein. This cDNA sequence is herein designated DNA13242 (Figure 130; SEQ ID NO:323). Based on the sequence homology, oligonucleotide probes were generated from the sequence of the DNA13242 molecule and used to screen a human placenta (LJB89) library prepared as described in paragraph 1 of Example 2 above. The cloning vector was pRK5B (pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)), and the cDNA size cut was less than 2800 bp.

The oligonucleotide probes employed included:

forward PCR primer 5'-GTGCAGCAGAGTGGCTTACA-3' (SEQ ID NO:326)

reverse PCR primer 5'-ACTGGACCAATTCTTCTGTG-3' (SEQ ID NO:327)

hybridization probe

5'-GATATTCTAGCATATTGTCAGAAGGAAGGATGGTGCAAATTAGCT-3' (SEQ ID NO:328)

A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 14-16 and ending at the stop codon found at nucleotide positions 446-448 (Figure 128; SEQ ID NO:321). The predicted polypeptide precursor is 144 amino acids long, has a calculated molecular weight of approximately 16,699 daltons and an estimated pI of approximately 5.6. Analysis of the full-length PRO181 sequence shown in Figure 129 (SEQ ID NO:322) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 20, a putative type II transmembrane domain from about amino acid 11 to about amino acid 31 and other transmembrane domains from about amino acid 57 to about amino acid 77 and from about amino acid 123 to about amino acid 143. Clone UNQ155 (DNA23330-1390) has been deposited with ATCC on April 14, 1998 and is assigned ATCC deposit no. 209775.

Analysis of the amino acid sequence of the full-length PRO181 polypeptide suggests that it possesses significant sequence similarity to the cornichon protein, thereby indicating that PRO181 may be a novel cornichon homolog. More specifically, an analysis of the Dayhoff database (version 35.45 SwissProt 35) evidenced significant homology between the PRO181 amino acid sequence and the following Dayhoff sequences, AF022811_1, CET09E8_3, S64058, YGF4_YEAST, YB60_YEAST, EBU89455_1, SIU36383_3 and PH1371.

EXAMPLE 51: Isolation of cDNA Clones Encoding Human PRO195

A cDNA sequence was isolated in the amylase screen described in Example 2 above and is herein designated DNA13199 (Figure 134; SEQ ID NO:332). The DNA13199 sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., *Methods in Enzymology* 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington,

Seattle, Washington; <http://bozeman.mbt.washington.edu/phrap.docs/phrap.html>). The consensus sequence obtained therefrom is herein designated as DNA22778.

Based on the DNA22778 sequence, oligonucleotide probes were generated and used to screen a human placenta library (LIB89) prepared as described in paragraph 1 of Example 2 above. The cloning vector was pRK5B (pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)), and the cDNA size cut was less than 2800 bp.

PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-ACAAGCTGAGCTGCTGTGACAG-3' (SEQ ID NO:333)

reverse PCR primer 5'-TGATTCTGGCAACCAAGATGGC-3' (SEQ ID NO:334)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the DNA22778 sequence which had the following nucleotide sequence

hybridization probe

5'-ATGGCCTTGGCCGAGGTTCTGGGGACCGCTTCGGCTGAAG-3' (SEQ ID NO:335)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO195 gene using the probe oligonucleotide and one of the PCR primers.

A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 70-72 and ending at the stop codon found at nucleotide positions 1039-1041 (Figure 132; SEQ ID NO:330). The predicted polypeptide precursor is 323 amino acids long, has a calculated molecular weight of approximately 36,223 daltons and an estimated pI of approximately 5.06. Analysis of the full-length PRO195 sequence shown in Figure 132 (SEQ ID NO:330) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 31, a transmembrane domain from about amino acid 241 to about amino acid 260 and a potential N-glycosylation site from about amino acid 90 to about amino acid 93. Clone UNQ169 (DNA26847-1395) has been deposited with ATCC on April 14, 1998 and is assigned ATCC deposit no. 209772.

Analysis of the amino acid sequence of the full-length PRO195 polypeptide suggests that it possesses no significant sequence similarity to any known protein. However, an analysis of the Dayhoff database (version 35.45 SwissProt 35) evidenced some degree of homology between the PRO195 amino acid sequence and the following Dayhoff sequences, P_P91380, AF035118_1, HUMTROPES_1, NUOD_SALTY and E70002.

EXAMPLE 52: Isolation of cDNA Clones Encoding Human PRO865

A cDNA sequence isolated in the amylase screen described in Example 2 above was herein designated DNA37642 (Figure 137, SEQ ID NO:338). The DNA37642 sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) to identify homologies therebetween.

The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington;

http://bozeman.mbt.washington.edu/phrap.docs/phrap.html). The consensus sequence obtained is herein designated DNA48615.

Based on the DNA48615 consensus sequence, probes were generated and used to screen a human fetal kidney (LIB227) library prepared as described in paragraph 1 of Example 2 above. The cloning vector was pRK5B (pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)), and the cDNA size cut was less than 2800 bp.

PCR primers (forward and reverse) were synthesized:

forward PCR primer 1 5'-AAGCTGCCGAGCTGCAATG-3' (SEQ ID NO:339)

forward PCR primer 2 5'-TTGCTTCTTAATCCTGAGCGC-3' (SEQ ID NO:340)

forward PCR primer 3 5'-AAAGGAGGACTTTCGACTGC-3' (SEQ ID NO:341)

reverse PCR primer 1 5'-AGAGATTTCATCCACTGTCCAAGTCG-3' (SEQ ID NO:342)

reverse PCR primer 2 5'-TGTCCAGAAACAGGCACATATCAGC-3' (SEQ ID NO:343)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA48615 sequence which had the following nucleotide sequence

hybridization probe

5'-AGACAGCGGCACAGAGGTGCTTCTGCCAGGTTAGTGGTTACTTGGATGAT-3' (SEQ ID NO:344)

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO865 gene using the probe oligonucleotide and one of the PCR primers.

A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 173-175 and ending at the stop codon found at nucleotide positions 1577-1579 (Figure 135; SEQ ID NO:336). The predicted polypeptide precursor is 468 amino acids long, has a calculated molecular weight of approximately 54,393 daltons and an estimated pI of approximately 5.63. Analysis of the full-length PRO865 sequence shown in Figure 136 (SEQ ID NO:337) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 23, potential N-glycosylation sites from about amino acid 280 to about amino acid 283 and from about amino acid 384 to about amino acid 387, a potential amidation site from about amino acid 94 to about amino acid 97, glycosaminoglycan attachment sites from about amino acid 20 to about amino acid 23 and from about amino acid 223 to about amino acid 226, an aminotransferase class-V pyridoxyl-phosphate amino acid sequence block from about amino acid 216 to about amino acid 222 and an amino acid sequence block similar to that found in the interleukin-7 protein from about amino acid 338 to about amino acid 343. Clone UNQ434 (DNA53974-1401) has been deposited with ATCC on April 14, 1998 and is assigned ATCC deposit no. 209774.

Analysis of the amino acid sequence of the full-length PRO865 polypeptide suggests that it possesses no significant sequence similarity to any known protein. However, an analysis of the Dayhoff database (version 35.45 SwissProt 35) evidenced some degree of homology between the PRO865 amino acid sequence and the following Dayhoff sequences, YMN0_YEAST, ATFCA4_43, S44168, P_W14549 and RABTCRG4_1.

EXAMPLE 53: Isolation of cDNA Clones Encoding Human PRO827

A cDNA sequence isolated in the amylase screen described in Example 2 above was found, by BLAST